b) detecting a sequence difference with respect to a reference sequence in the subset of nucleic acid molecules,

D3

wherein steps (a) and (b) enrich for and identify a nucleic acid sequence difference with respect to a reference sequence.

159. (New) The method of claim 1 wherein said subset of nucleic acid molecules having a sequence that binds to said sequence-specific binding activity comprises less than every molecule in the population of nucleic acid molecules in said sample.

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REMARKS

Upon entry of the amendments directed herein, claims 1-3, 57-74 and 145-159 are pending. Applicants note that there is a discrepancy between the pending claims acknowledged in the Final Office Action mailed September 6, 2001 (Paper No. 13) and the pending claims filed. Specifically, the Final Office Action listed claims 1-3, 57-74 and 145-156 as pending, while new claims 157 and 158, submitted in response to the non-final Office Action mailed February 28, 2001 (Paper No. 8), were not entered. New claims 157 and 158 were submitted on August 28, 2001 in the response to the non-final Office Action, and therefore should have been entered as of right. Applicants believe that claims 157 and 158 are patentably distinguished over the prior art cited, particularly by the recitation of "contacting a nucleic acid sample with a DNA binding domain of a restriction endonuclease that cleaves said nucleic acid sample 300,000 times or fewer." Applicants respectfully request acknowledgement of entry of these claims, and their consideration by the Examiner at this time.

New claim 159 set forth above is supported in the specification at page 29, line 20 to page 30, line 2. The new claim, which is dependent from claim 1, more precisely sets out the method of one embodiment of the invention with regard to what is meant by the term "subset of nucleic acid molecules having a sequence that binds to said sequence-specific binding activity."

Amendment of claim 157:

In the Advisory Action mailed March 19, 2002 (Paper No. 19), the Examiner noted that previously non-considered claim 157 would be objected to for recitation of the phrase "for fewer," which rendered the claim incomplete. Applicants have amended claim 157 herein in order to correct the typographic error. The phrase now reads "or fewer," thereby alleviating the problem.

Rejections over prior art:

Claims 1-3 and 150-153 are rejected under 35 U.S.C. §102(a) as being anticipated by Oefner et al. (U.S. Patent No. 5,795,976). Claims 1-3, 57-74 and 145-155 are rejected under 37 C.F.R. §103(a) as being obvious over Oefner et al. in view of Bloch et al. (U.S. Patent No. 5,866,429), and claims 1-3, 57-74 and 145-156 are rejected as obvious over Oefner et al. in view of Bloch et al. and Fox et al. (U.S. Patent No. 6,140,086). Thus, all claims are rejected as anticipated or rendered obvious by Oefner et al., either alone or in combination, respectively.

Applicants submit that all independent claims require that the sample comprises a **subset** of nucleic acid molecules. Claims 1-3 and 150-153 require that the "sample comprises a subset of nucleic acid molecules having a sequence that binds said sequence specific binding activity." Applicants submit that Oefner et al. does not teach a sample comprising such a subset. A "subset," both as the term is commonly understood in the language and as defined in the specification (page 29, line 20 to page 30, line 2), *comprises less than every molecule in the population*. Oefner et al. teaches mixing two samples of nucleic acids to be compared, denaturing and re-annealing them, followed by denaturing HPLC to identify sequence differences, such as mismatches. Applicants stress that in order for the Oefner et al. methods to work, hybrids must be formed between strands with perfect complementarity and between strands with mismatches. The denaturing HPLC method of the Oefner et al. disclosure detects differences in elution profiles between hybrid molecules with perfect complementarity and hybrid molecules with sequence differences. Thus, if one strand of a molecule according to Oefner et al. is considered, for the sake of argument alone, to be a "sequence specific binding activity," the fact that both fully complementary and mismatched nucleic acid strands in the sample

form hybrids with that strand (i.e., *bind* the sequence specific binding activity) means that the sample does not "comprise a subset of nucleic acid molecules having a sequence that binds said sequence specific binding activity." That is, every molecule in the annealed population has a sequence that binds the sequence specific binding activity, so there is no subset having a sequence that binds the sequence specific binding activity. Therefore, claims 1-3 and 150-153, which require such a subset, are novel over Oefner et al. Applicants respectfully request the withdrawal of the §102 rejection of these claims over Oefner et al.

Claims 1-3, 57-74 and 145-155 are rejected under 35 U.S.C. §103(a) as obvious over Oefner et al. in view of Bloch et al. With regard to claim 1 and dependent claims 2-1-3 and 145-155, Applicants submit that, as discussed above, Oefner et al. does not teach or suggest a "subset of nucleic acid molecules having a sequence that binds to said sequence specific binding activity," as required by claim 1. Because the Oefner et al. reference does not teach a subset, it does not teach or suggest that "a bound subset of nucleic acid molecules is retained by said sequence specific binding activity" as required by claim 1. Applicants submit that neither Bloch et al. nor Fox et al. teaches or suggests a bound subset of nucleic acid molecules retained by the sequence specific binding activity. Claims 145-149 and claims 154-155, which all ultimately depend from claim 1, are similarly not satisfied by the combination of references because the combination lacks the necessary teaching of a subset. Because Oefner et al. and Bloch et al. thus do not teach or suggest all of the required elements, claim 1 and claims 2, 3, and 145-156 that depend from it are not obvious over this combination of references.

Similarly, claims 1-3, 57-74 and 145-156 are rejected over Oefner et al. in view of Bloch et al. and Fox et al. The Examiner cites Fox et al. as teaching NotI digestion. Applicants submit that Fox et al. does not remedy the deficiency of Oefner et al. and Bloch et al. with regard to the subset of nucleic acid molecules having a sequence that binds to said sequence specific binding activity," or the limitation that "a bound subset of nucleic acid molecules is retained by said sequence specific binding activity" as required by claim 1. Therefore, claim 1 and claims 2, 3 and 145-156 are not obvious over the combination of Oefner et al. and Bloch et al. and Fox et al.

With regard to claim 57 and its dependents, Applicants submit that Oefner et al. does not teach the recited steps in the order recited in the claim as amended. The order of steps recited in the amendment is supported in the specification at page 12, line 17 to page 13, line 4 (step (b) before step (c)), and at page 42, line 20 to page 43, line 6 (step (c) before step (b)). In the claim as amended, the fragmenting step (a) is followed by either "physically separating" then "operatively linking" ((b) then (c)), or "operatively linking" then "physically separating" ((c) then (b)).

The Examiner acknowledged that Oefner et al. does not teach the recited steps in the order recited, but cites *In re Gibson*, 39 F.2d 975, 5 U.S.P.Q. 230 (CCPA 1930) as standing for the proposition that the "order of mixing" is prima facie obvious. Applicants submit first that there is a dramatic difference between the "order of mixing" in a synthetic scheme for making brake pads at issue in *Gibson*, and the order of performing steps in a method as claimed. The order in which specific method steps according to the invention are carried out will influence not only the ultimate result of the steps, but also whether certain of the steps *are needed at all*, or put differently, whether there is motivation to combine the individual steps cited in the order suggested by the Examiner. Thus, a reference that may provide individual steps that meet the elements of a claimed method does not automatically render obvious the method that combines those steps in a particular fashion for a particular result. This is particularly so for the steps of claim 57.

The teachings construed by the previous Office Action to satisfy the recited steps of claim 57 do not support their assembly into the order required by claim 57. In fact, the reference provides no motivation to perform, in one method, all of the steps cited as corresponding to recited elements of claim 57. For example, if one performs the denaturing HPLC taught in Example 2 of Oefner et al., which was cited by the Examiner as satisfying claim 57 step (b), "physically separating a subset of said nucleic acid fragments based on the size of the fragments," the aim of Oefner's method, i.e., determining a sequence difference between two molecules, is achieved without the need for additional steps taught elsewhere in the reference in regard to other methods. Oefner et al. does not teach "operatively linking" denaturing HPLC-separated species generated in Example 2 or elsewhere in the reference to an oligonucleotide or a vector, nor does the

reference teach a subsequent step of "replicating the operatively linked subset to form an enriched collection of replicated molecules" as required by claim 57. In fact, there is no reason to perform such additional steps after the "separation" (HPLC) step, because that separation step itself provides the desired end result of the Oefner method, i.e., a determination that two molecules examined have a sequence difference with respect to one another. According to claim 57, "operatively linking" and "replicating" steps must necessarily follow the physical separation step. However, if a sequence difference is determined by the denaturing HPLC separation step of Oefner et al., it would not be obvious to then perform additional steps, even if they are mentioned elsewhere in the document in relation to other methods, because the desired end result, i.e., sequence difference determination, has already been achieved,. Therefore, the method of claim 57 is not rendered obvious by Oefner et al.'s Example 2 in view of other passages cited.

Similarly, in column 13, line 21 to column 17, line 12 of Oefner et al. (also cited in the previous Office Action) there is no step of "physically separating a subset of said nucleic acid fragments based on the size of the fragments" followed by a step of "operatively linking" the subset of size-separated fragments to "an oligonucleotide or vector." This is because the only step of physically separating that is taught is the denaturing HPLC step that determines the sequence difference. Once that step has been performed, the desired end result has been achieved, and there is no motivation or need to perform other steps according to the Oefner et al. method. Therefore, it would not be obvious to perform the subsequent "operatively linking" or "replicating" steps called for in the method of claim 57 after the "physically separating" step.

Claim 69 and its dependents are also included under the §103 rejection over Oefner et al. in view of Bloch et al., as well as the rejection in view of Oefner et al. in view of Bloch et al. and Fox et al. Applicants submit that Oefner et al. does not teach or suggest fragmenting a genomic nucleic acid sample from one or more individuals before physically separating a subset of those fragments based on the size of the fragments. Further, while Bloch et al. may teach cleavage of fragments with a restriction endonuclease, Bloch et al. does not teach or suggest "fragmenting a genomic nucleic acid sample from one or more individuals" as called for in claim 69 as amended.

The Bloch et al. reference teaches that:

> "Another preferred method, used alone or together with PCR, for providing nucleic acid suitable for anion-exchange HPLC analysis is digestion with a restriction endonuclease, a procedure which, for relatively homogeneous DNA, generates *a finite and often low number of well defined fragments*." (Column 13, lines 19-24; emphasis added).

Applicants submit that if one applies the teaching of Bloch et al., regarding restriction endonuclease cleavage of a sample, to a genomic sample as called for in claim 69 as amended, one will not obtain the "low number of fragments" taught as the goal of such digestion in the Bloch et al. reference. That is, Bloch et al. teaches away from a method in which many fragments are generated.

With regard to the number of restriction endonuclease-generated fragments desired, Bloch et al. teaches first, that:

> "Chromatographic analysis of nucleic acids is beneficially simplified if the test sample *contains relatively few different and distinct nucleic acid species*, so that the elution profile consists of *easily resolvable and identifiable peaks standing out above a low background* of W absorbance." (Column 12, lines 60-65; emphasis added)

That is, the desire for a low number of well defined fragments expressed at column 13, lines 19-24 (copied above) is not simply a preferred embodiment, but reflects a limitation in the method due to problems in resolving larger numbers of fragments over the increased background that accompanies the larger numbers.

Second, the desire for low numbers of fragments is exemplified in the passage, cited in the Office Action, where Bloch et al. teaches:

> "The double-stranded DNA sample was an endonuclease HaeIII digest of the plasmid pBR322 (Boehringer Mannheim), supplied in pH 8.0, 10 mM Tric-Cl, and 1 mM EDTA, diluted 1/5 into 10 mM Tris-Cl, 50 mM KCl, pH 8.3. It includes blunt-ended fragments of the following molecular sizes in base pairs: 51, 57, 64, 80, 89, 104, 123, 124, 184, 192, 213, 234, 267, 434, 458, 504, 540, and 587." (Column 19, lines 58-64)

That is, in the only example of endonuclease cleavage in the Bloch et al. reference, there were only 18 fragments generated. This stands in stark contrast to the number of fragments generated when a genomic DNA sample is cleaved with a restriction endonuclease. For example, even AscI, which recognizes an 8 base GC-rich sequence and results in an average fragment size of 670,000 base pairs, generates approximately 4900 fragments upon cleavage of the human genome (genome size = $3.3 \times 10^9 / 6.7 \times 10^5$ bp average AscI fragment size in human genome (New England Biolabs Website reference appendix – www.neb.com)). Applicants submit that 4900 different and distinct fragments is far from the "relatively few different and distinct" fragments taught by Bloch et al. The same is true of cleavage of, for example, human genomic DNA with the 8-cutter NotI, which generates approximately 330,000 fragments of 100,000 bp average size. 330,000 fragments is clearly not a low number of fragments, especially when considered relative to the highest number exemplified by Bloch et al., which was 18.

Finally, the Bloch et al. reference also teaches the use of PCR to generate fragments for anion-exchange HPLC analysis. See for example, column 13, lines 1-5, which refer to PCR as "a method of greatly amplifying the number of molecules of *one or a few specific nucleic acid sequences*, most commonly in the size range of 50 to 1,000 bp, which is perfectly suited for anion-exchange HPLC separation on the basis of size" (emphasis added). Lines 8-11 of column 13 refer to PCR as tending "to generate *just one or a few DNA fragments* in exactly the concentration range needed for UV absorbance detection of chromatographic peaks" (emphasis added). The reference thus further stresses that *few* fragments are a requirement of the anion-exchange HPLC method taught.

In view of the above, Applicants submit that Bloch et al. does not remedy the Oefner reference's lack of a teaching of fragmenting a genomic nucleic acid sample prior to physically separating a subset of the nucleic acid fragments based on the size of the fragments. Not only is there no teaching of fragmenting genomic DNA in either reference, but Bloch et al. actually teaches away from such fragmenting due to a limitation on the number of different fragments that can be resolved in the anion-exchange method taught in that reference. Therefore, the combination of Bloch et al.

with Oefner et al. does not teach or suggest all limitations of claim 69 as amended. Applicants respectfully request the withdrawal of the §103 rejection of claim 69 and its dependents over Oefner et al. in view of Bloch et al.

With regard to the Fox et al. reference, Applicants submit that the addition of NotI cleavage taught by Fox et al. does not remedy the deficiency of Oefner et al. and Bloch et al. with regard to the step of fragmenting a genomic nucleic acid sample from one or more individuals recited in claim 69 as amended. While Fox et al. teaches cleavage with NotI (among other restriction endonucleases), this does not overcome the fact that Bloch et al. does not teach, and actually teaches away from, fragmenting a genomic nucleic acid sample. Applicants therefore submit that claim 69 and its dependents are not obvious over a combination of Oefner et al., Bloch et al., and Fox et al. Applicants respectfully request the withdrawal of the §103 rejection of claims 69-74 over these references.

In view of the above, Applicants submit that claims 1-3, 57-74 and 145-158 are neither anticipated by nor obvious over any combination of the cited art. Applicants submit that all issues raised in the Office Action have thus been addressed herein. Reconsideration of the claims is respectfully requested.

Respectfully submitted,

5/2/02
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Version of amendments marked to show changes:

57. (Twice amended) A method of enriching for and identifying nucleic acid sequence differences with respect to a reference sequence comprising:

a) fragmenting a nucleic acid sample from one or more individuals;

b) physically separating a subset of said nucleic acid fragments based on the size of the fragments;

c) operatively linking a fragment created in step (a) or said subset [of] created in step (b) with an oligonucleotide or a vector;

d) replicating said operatively linked subset to form an enriched collection of replicated molecules; and

e) detecting one or more nucleotide sequence differences in the members of said collection of step (d) with a method that detects one or more nucleotide differences with respect to a reference sequence,

wherein said steps (b) and (c) follow step (a) but can occur in either order, followed thereafter by steps (d) and (e) in that order, wherein said steps (a)-(e) enrich for and identify a nucleic acid sequence difference with respect to a reference sequence.

69. (Twice Amended) A method of enriching for and identifying nucleic acid sequence differences with respect to a reference sequence comprising:

a) fragmenting a genomic nucleic acid sample from one or more individuals;

b) physically separating a subset of said nucleic acid fragments based on the size of the fragments;

c) detecting one or more nucleic acid sequence differences with respect to a reference sequence in the members of said separated molecules of step (b), wherein steps (a)-(c) enrich for and identify a nucleic acid sequence difference with respect to a reference sequence.

157. (Amended) A method of enriching for and identifying a nucleic acid sequence difference with respect to a reference sequence comprising:

a) contacting a nucleic acid sample with a DNA binding domain of a restriction endonuclease that cleaves said nucleic acid sample 300,000 times [for] or fewer, wherein said sample comprises a subset of nucleic acid molecules having a sequence that is bound by said DNA binding domain, and wherein a bound subset of nucleic acid molecules is retained by said DNA binding domain, such that the subset of bound nucleic acid molecules is enriched for molecules comprising the sequence recognized by said DNA binding domain; and

b) detecting a sequence difference with respect to a reference sequence in the subset of nucleic acid molecules,

wherein steps (a) and (b) enrich for and identify a nucleic acid sequence difference with respect to a reference sequence.

159. (New) The method of claim 1 wherein said subset of nucleic acid molecules having a sequence that binds to said sequence-specific binding activity comprises less than every molecule in the population of nucleic acid molecules in said sample.